Title: BioBricking logic gate #1

Goal: To biobrick the logic gate
Members that worked on project: Daren Kraft

Summary: The logic gate consists of a strong Anderson promoter upstream of a terminator. The terminator is flanked by the Bxb1 invertase recognition sites. We were successful in putting this gate onto a pSB1C3 backbone.

New part number: BBa_K1718002
Part size: 293bp

PCR:

- Purpose: to amplify the gate off of a gblock
- PCR polymerase: Q5
- PCR of: BBa_J04450

Reagent	**50 ul reaction**	**Concentration**
5x Q5	10 ul	1 x
10 mM dNTPs	1 ul	200 uM
10 uM F. Primer	2.5 ul	0.5 uM
10 uM R. Primer	2.5 ul	0.5 uM
DNA	1ul	0.5ng
Q5 polymerase	0.5 ul	0.02 U/ul
Water	22.5 ul	

Reaction conditions:

Step	Temp (C)	Time
I. Denature	98	5 min.
35 cycles	98	10 s
	62	30 s
	72	30 s
72	1ul	2 min.

- Primers used: GEM025

PCR clean-up:

- Elution volume: 30ul
- Followed the kit instructions. There were no incidences.

Digestion:

- State purpose: To cut the linearized PCR product of the gate in order to insert it onto a pSB1C3 backbone.
- Digestion of: the gate PCR product

Reagent	**50 ul reaction**	**Concentration**
Cutsmart Buffer	5 ul	1 x
EcoRI-HF	1 ul	10 units
PstI-HF	1 ul	10 units
DNA	8 ul	500 ng
Water	35 ul	

- Digestion of: pSB1C3

Reagent	**50 ul reaction**	**Concentration**
Cutsmart Buffer	5 ul	1 x
EcoRI-HF	1 ul	10 units
PstI-HF	1 ul	10 units
DNA	3 ul	500 ng
Water	40 ul	

Parts were the correct size on the gel
Gel extracted the gate and backbone

Ligation:

- Used the logic gate and the pSB1C3 backbone, both digested with E and P
- 20ul reaction incubated overnight at 16C

Transformation:

- Heat shocked at 42C for 30s.
- Cells were then recovered in 900ul SOC for 1hr.
- Cells were spun down then resuspended in 200ul and plated onto Chlor plates (concentration of 170) and were allowed to grow overnight

Overnight:

- Three colonies grew. An overnight was made from each (5mL LB with Chlor at a concentration of 170).

Mini-prep:

- Samples were mini-prepped following the Qiagen mini-prep kit.

Digestion:

- State purpose: to check insert size of overnights
- Digestion of: gate on pSB1C3

Reagent	**20 ul reaction**	**Concentration**
Cutsmart Buffer	2 ul	1 x
EcoRI-HF	.4 ul	4 units
PstI-HF	.4 ul	4 units
DNA	3 ul	200 ng
Water	14.2 ul	

Gel:

- Latter: 2-log
- The pSB1C3 backbone should be 2070bp
- The gate should be about 300bp

Initial characterization: On the gel, the insert was of the correct size. As there was no reported on this part, we could not do any further characterization.
Next step: We will add RFP behind the gate in order to test leakiness in the absence of Bxb1 and functionality in the presence of Bxb1.

Sequence:

- Primers: V2F
- Result: The sequence was as expected.

Part submission:

- New part name: Genetic Switch for Bxb1 invertase
- New part number: BBa_K1718002